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A Conditional Suicide System in *Escherichia coli* Based on the Intracellular Degradation of DNA

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The potential risks associated with the intentional or unintentional release of genetically engineered microorganisms led to the construction of biological containment systems by which bacteria are killed in a controlled suicide process. In previously published suicide systems, cell killing was caused by proteins destroying the cell membrane or cell wall. Here a conditional cell killing system based on the intracellular degradation of cellular DNA is presented. The nuclease gene used was that of the extracellular nuclease of *Serratia marcescens*. The nuclease gene was deleted for the leader-coding sequence, and the truncated gene was put under the control of the lambda p_L promoter. Following thermoinduction of the nuclease gene cassette in *Escherichia coli*, cell survival dropped to 2×10^{-5}, and more than 80% of the radioactively labeled DNA was converted to acid-soluble material within 2.5 h in the absence of cell lysis. The majority (84%) of clones which survived thermoinduced killing turned out to be as sensitive to a second thermoinduction as the original strain. The other clones showed somewhat slower killing kinetics or slightly higher final levels of survivors. The suicide system described combines the regulated killing of cells with the destruction of intracellular DNA otherwise potentially available for horizontal gene transfer processes.

The use of genetically engineered microorganisms (GEMs) in closed systems involves the possibility of accidental releases of these cells into the environment. Moreover, both the planned and the already performed applications of GEMs in agriculture, waste treatment, and the production of certain raw materials rely on the release of great quantities of cells into the environment. The main concern about the release of transgenic organisms comes from the uncertainty as to how these organisms will behave in the environment (32). There is the risk of unanticipated survival and reproduction in the environment, with negative ecological effects. Another problem is the uncontrolled transfer of genetically engineered DNA to other organisms in the environment. Among bacteria, such horizontal gene transfer can proceed by various processes, such as conjugation, transduction, and transformation in natural microbial habitats (16; for review, see reference 30). Therefore, as a biological containment strategy, conditional suicide systems for bacteria have been designed and constructed to cause the controlled death of cells (for review, see reference 20). Such suicide systems consist of a regulated killing gene which is expressed in response to specific factors in the environment or in closed systems. The killing genes successfully used so far in suicide systems determined the synthesis of cell-lysing agents, including membrane-destabilizing polypeptides (7, 15, 21) and cell-destroying levane (24) and lysozyme (28). These genes were put under the control of promoters inducible by increased temperature, starvation, or the presence of certain chemicals (11, 21, 28). However, the use of membrane-destroying agents for the killing of cells possibly facilitates the release of recombinant DNA into the surrounding milieu. Since experimental data suggest that free DNA can persist in nonsterile soils for weeks or months (25, 26) and is available for uptake by bacterial cells that are naturally transformable, it is assumed that released DNA can be disseminated among bacteria in the environment by genetic transformation (17). Therefore, a bacterial suicide system combining controlled killing of cells with the destruction of genetic material before its release from cells is desirable in order to limit both the survival of GEMs and the transfer of recombinant DNA to other organisms.

In this communication, a conditional suicide system in which cell death is caused by the controlled derepression of a nuclease gene that results in the degradation of intracellular DNA to acid-soluble material is presented. For these experiments, the *nuc* gene of *Serratia marcescens* was chosen (2), specifying the extracellular nuclease of *S. marcescens* (hereafter referred to as the *Serratia* nuclease) which endonucleolytically cleaves RNA and DNA to acid-soluble material (22) and introduces single- and double-stranded cuts into duplex DNA (1).

MATERIALS AND METHODS

Bacterial strains and plasmids. Table 1 contains descriptions of the bacterial strains and plasmids used in this study. *Escherichia coli* AH1 was constructed by P1 transduction (19) with *E. coli* JC10289(pKY102) as the donor of the *recA* deletion (13).

Molecular techniques. To inhibit the action of the *Serratia* nuclease during plasmid preparation from cells overproducing the enzyme, the method of Birnboim and Doly (6) was applied with the following modifications. (i) Before lysis, the cells of each culture were washed in half a volume of 10 mM NaCl to remove extracellular nuclease. (ii) During alkaline lysis, all incubation times were shortened from 30 or 60 min (6) to 5 min and all centrifugations were done at 4°C. (iii) After precipitation of DNA with ethanol, the pellet was resuspended in 10 mM Tris-HCl (pH 8.0) with 100 mM EDTA. (iv) After the addition of NH_4-acetate and before the second ethanol precipitation, the mixture was incubated for 15 min at 70°C and for 10 min on ice and then centrifuged (12 min at 13,000 × *g* and 4°C). (v) The supernatant was extracted twice with phenol-chloroform-isoamyl alcohol (25:24:1) and once with chloroform-isoamyl alcohol (24:1) and then precipitated with etha-

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TABLE 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Description	Source or reference
Strains		
E. coli TGE900	F′ *sup*$^+$ *ilv bio*(λ*c*I857Δ*Bam*HI)	8
E. coli AH1	TGE900 Δ(*recA-srl*)306::Tn*10*, Tc^r	This work
Plasmids		
pET81F$^+$	Ap^r, 2,730 bp; expression vector with the T7 Φ10 promoter, ribosome-binding site, and ATG start codon	31
pSF1	Ap^r, 4,820 bp; contains the *ssb* gene of *E. coli* under the control of the lambda p_L promoter	4
pSF1E	Ap^r, 4,149 bp; pSF1 was digested with *Eco*RI for deletion of the *ssb* gene and religated	This work
pNuc4	Ap^r, 4,128 bp; gene for the extracellular nuclease of *S. marcescens* SM6 (*nuc*) in vector pUC18	2
pAH10	Ap^r, 3,660 bp; leader-free nuclease gene of *S. marcescens* (*nuc*ΔL) under the control of the T7 Φ10 promoter in vector pET81F$^+$	This work
pAH12	Ap^r, 5,227 bp; leader-free nuclease gene of *S. marcescens* (*nuc*ΔL) under the control of the lambda p_L promoter in vector pSF1E	This work

nol. For cloning experiments, plasmid DNA was isolated with the Qiaprep-spin plasmid kit (Diagen, Düsseldorf, Germany) and extracted twice with phenol-chloroform-isoamyl alcohol as described above. The normal Qiagen purification system (plasmid minikit) plus phenol extraction was ineffective in removing the *Serratia* nuclease.

Cloning experiments were done by standard procedures (18). *E. coli* was transformed by electroporation (Gene pulser; Bio-Rad, Munich, Germany) (25 μF, 12.5 kV cm^{-1}, and 200 Ω).

Cloning of the *nuc* gene deleted for the leader peptide-coding nucleotide sequence. The DNA fragment coding for the mature *Serratia* nuclease was cut from the plasmid pNuc4 (2) by digestion with *Eag*I and *Bss*HII (Fig. 1). After mung bean nuclease digestion, the fragment was joined to a start codon in the vector pET81F$^+$ (31) to give pAH10 (Fig. 1). pET81F$^+$ was prepared for cloning by digestion with *Nco*I and *Bam*HI and by filling in the resulting single-stranded ends of the vector with the Klenow fragment of DNA polymerase I of *E. coli*. The truncated nuclease gene (termed *nuc*ΔL) together with the ribosome-binding site and the start codon of pET81F$^+$ was cut out of pAH10 and cloned downstream of the lambda p_L promoter of the pBR322-derived plasmid pSF1 (4) into an *Eco*RI site by blunt-end ligation (Fig. 1). For this, pAH10 was digested with *Ssp*I, *Bam*HI, and mung bean nuclease; pSF1 was digested with *Eco*RI and mung bean nuclease; and the ligation mixture was used to transform *E. coli* TGE900. To screen for transformants which contained an active suicide cassette (*nuc*ΔL gene downstream of the lambda p_L promoter), clones obtained at 28°C were streaked on Luria-Bertani (LB) agar plus ampicillin (100 μg ml^{-1}) and incubated at 28°C; for thermoinduction of the lambda p_L promoter, they were incubated at 42°C. Clones having an active suicide cassette grew only with incubation at 28°C. Among the 40 Ap-resistant transformants screened, 12 were thermosensitive for growth. From the thermosensitive clones, plasmid DNA was isolated and analyzed by restriction mapping. One of the plasmids which had the inserted nuclease cassette in the correct orientation relative to the p_L promoter was termed pAH12 (Fig. 1).

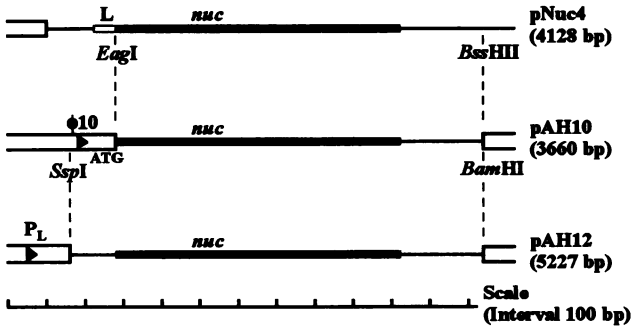

FIG. 1. Construction of the containment plasmid pAH12 (for details, refer to Materials and Methods). L, nucleotide sequence of the leader peptide; *nuc*ΔL, coding sequence for the mature *Serratia* nuclease; Φ10, T7 Φ10 promoter; p_L, lambda p_L promoter; ATG, start codon. Boxes represent vector DNA. Lines represent insert DNA. Arrowheads indicate the locations and directions of promoters.

Determination of survival after thermoinduction of killing. Cells were grown in LB broth (9) supplemented with ampicillin (100 μg ml^{-1}) at 28°C to approximately 2 × 10^8 cells ml^{-1} (log-phase cells). The culture was divided into two equal parts. One part was further incubated at 28°C, and the other part was incubated at 42°C for thermoinduction of the lambda p_L promoter. At various time intervals, viable counts were determined by serial dilution and plating on LB agar with ampicillin (100 μg ml^{-1}). Plates were incubated for 24 h at 28°C. Survival (n/n_0) is the viable count of the culture after the indicated times (n) divided by the viable count of the culture before induction (n_0).

Intracellular DNA degradation. DNA was labeled by the growth of cells in LB broth plus ampicillin (100 μg ml^{-1}), 2′-deoxyadenosine (250 μg ml^{-1}), and [*methyl*-^{3}H]thymidine (1.5 × 10^5 Bq ml^{-1}; specific activity, 925 GBq mmol^{-1}) for three generations at 28°C. At about 2 × 10^8 cells ml^{-1}, cells were collected by filtration, washed in 30 ml of prewarmed phosphate buffer, and suspended in 30 ml of prewarmed LB broth plus ampicillin (100 μg ml^{-1}). The culture was divided into three equal parts and incubated further at 28°C. After 30 min, one culture was supplemented with chloramphenicol (100 μg ml^{-1}) to inhibit protein expression and was incubated for 30 min at 42°C for thermoinduction of the lambda p_L promoter. The second culture was treated in the same manner except that chloramphenicol was omitted. After thermoinduction, the incubation of these two cultures was continued at 28°C. The third culture was incubated at 28°C throughout the experiment. After various time periods, 0.2-ml samples of all three cultures were removed and cells were precipitated by the addition of 0.2 ml of ice-cold 12% trichloroacetic acid (TCA). After incubation on ice for at least 30 min, the precipitate was collected on glass fiber filters (GF102; Schleicher & Schuell, Dassel, Germany), washed with 3 ml of ice-cold TCA, and then rinsed twice for 2 min in baths containing 6% TCA. Then the filters were rinsed in ice-cold 70% ethanol and dried at 40°C for 14 h. Filters were placed into 10 ml of toluene-based

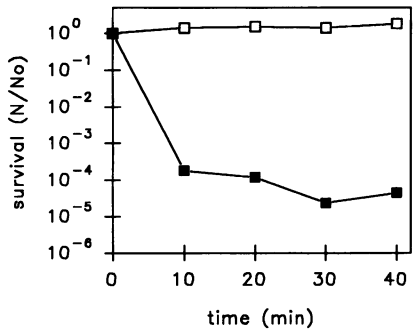

FIG. 2. Survival of *E. coli* TGE900 with the suicide plasmid pAH12 (■) or the vector pSF1E (□) after thermoinduction by incubation at 42°C (0 to 40 min). Viable counts were determined as described in Materials and Methods. Survival (n/n_0) is the viable count of the culture at the indicated times (n) divided by the viable count of the culture before induction (n_0). Data are the means of two (pSF1E) or three (pAH12) independent experiments.

scintillation fluid (Unisolve 1; Werner Zinsser Scintillators, Frankfurt, Germany), and TCA-insoluble radioactivity was determined with a Betamatic scintillation counter (Kontron, Eding, Germany).

RESULTS

Construction of the suicide cassette. The *nuc* gene coding for the *Serratia* nuclease determines a polypeptide of 266 amino acids whose 21 N-terminal amino acids constitute a leader peptide (5). The leader peptide is removed during secretion, which activates the mature nuclease (2, 5). The construction of the containment plasmid (pAH12) has been described above. This plasmid contains the nuclease gene of *S. marcescens* deleted for the leader-coding sequence (termed *nuc*ΔL) under the control of the lambda p_L promoter. In *E. coli* TGE900 (8), which carries the gene for the thermosensitive lambda *c*I857 repressor in the chromosome, the expression of the *nuc*ΔL gene is repressed at 28°C but can be thermally induced.

Thermoinduction of killing. Log-phase broth cultures of *E. coli* TGE900(pAH12) or TGE900(pSF1E) grown at 28°C were shifted from 28 to 42°C. At 42°C, the thermosensitive lambda *c*I857 repressor is rapidly inactivated, thereby derepressing transcription of the nuclease gene from the lambda p_L promoter. In a time course experiment (Fig. 2), the survival of cells was determined by plating at 28°C on LB agar with ampicillin following the heat pulse. The survival of *E. coli* TGE900(pAH12) but not that of TGE900 with the vector plasmid (control) declined rapidly after thermoinduction and reached its minimum after 30 min at 42°C (2.3×10^{-5}). Longer times at 42°C did not increase killing. A series of additional experiments (Table 2) led to the conclusion that the cell killing resulted specifically from the derepression of the nuclease gene in strain TGE900(pAH12). First, thermoinduction of TGE900(pSF1E) did not substantially affect growth during the 30-min induction period compared with that of the 28°C culture. Second, when the vector contained the *ssb* gene of *E. coli* coding for single-stranded DNA-binding protein, thermoinduced overproduction of this protein did not cause killing either. This suggests that the killing of cells with pAH12 by thermal treatment did not result merely from the derepression of an additional protein-coding gene on the plasmid. The slight decrease in survival following thermoinduced overproduction of SSB protein (Table 2) is in accord with a previous observation and probably results from interference of excess *ssb* protein with replication (23). Finally, a *recA* deletion was crossed into TGE900(pAH12) [producing AH1(pAH12)] to examine whether reduced DNA repair capacity of cells would disclose chromosomal DNA damage caused by low levels of *Serratia* nuclease expressed even in uninduced cells. The *recA*-dependent recombinational repair system repairs double-stranded breaks in *E. coli*, which makes *recA* mutants highly sensitive to DNA-breaking agents (27). Therefore, *recA* deficiency could result in a lower growth rate and increased sensitivity to thermoinduction. This, however, was not the case. The reduced growth of AH1(pAH12) at 28°C (Table 2) was also observed with AH1(pSF1E) and is typical for *recA* mutants compared with their *recA*$^+$ counterparts. Thermoinduced killing in the *recA* derivative was not enhanced (Table 2). Thus, nuclease expression from pAH12 in TGE900 appears to be very low or absent at 28°C. This conclusion is also supported by the observation that with repression of the p_L promoter (28°C), there is no difference in the growth rates of *E. coli* TGE900 with plasmid pAH12 (*nuc*ΔL), pSF1E (vector), or pSF1 (*ssb*) (Table 2). Also, in separate experiments, it was found that after 50 generations in LB at 28°C, 100% of the cells of *E. coli* TGE900(pAH12) and TGE900(pSF1E) were still ampicillin resistant, suggesting that the suicide cassette was effectively shut off at 28°C. The similar levels of killing of TGE900 (pAH12) and its *recA* counterpart, AH1(pAH12), upon thermoinduction (Table 2) suggest that the repair capacity of cells does not help against the killing effect of the derepressed nuclease.

TABLE 2. Survival of *E. coli* strains grown at 28°C with and without thermoinduction of the lambda p_L promoter (42°C)

Strain and description	Survival (n/n_0)[a]	
	42°C culture	28°C culture
TGE900(pAH12) (*nuc*ΔL)	2.3×10^{-5} ($\pm 1.6 \times 10^{-5}$)	1.5
TGE900(pSF1E) (vector)	1.2	1.5
TGE900(pSF1) (*ssb*)	0.8	1.5
AH1(pAH12) (*nuc*ΔL *recA*)	5.8×10^{-5} ($\pm 1.2 \times 10^{-5}$)	1.1
AH1(pSF1E) (vector, *recA*)	1.3	1.0

[a] Survival (n/n_0) is the viable count of culture after 30 min at 42 or 28°C (n) divided by the viable count of culture at time zero (n_0); for details, refer to Materials and Methods. Data are the means of two independent experiments; numbers in parentheses are the standard deviations of the means.

Examination of surviving clones. After the thermoinduction of killing, survival occurred at a low but significant level (about 2×10^{-5}). Since viable counts were determined by plating on LB agar with ampicillin, the growth of plasmid-free segregants was excluded as a cause for survival. Survival following thermoinduction was about 1 order of magnitude higher when LB without ampicillin was used for plating, indicating that slightly less than 0.1% of the segregants present in the cultures were plasmid free.

The phenotypes and genotypes of the clones obtained at the minimum of survival (30 min at 42°C; Fig. 2) were examined in order to find out whether survival was the result of transient resistance or mutation. Mutations could have occurred in the plasmid pAH12 (e.g., in the promoter/operator region or the *nuc*ΔL gene) or in the bacterial chromosome (e.g., in the lambda *c*I857 repressor gene reversing its temperature sensitivity). Clones which had survived heat induction were streaked on LB agar with ampicillin and incubated at 28 and 42°C. This procedure was repeated three times. Among the 25 clones

TABLE 3. Thermoinduced killing of *E. coli* TGE900(pAH12) clones which had survived a 42°C treatment[a]

Strain and mutant no.	Phenotype[b]	Survival (n/n_0) at:	
		30 min	60 min
TGE900(pAH12)	Sensitive	2.3×10^{-5}	7.8×10^{-5}
8	Slightly resistant	1×10^{-5}	8×10^{-6}
8[c]	Slightly resistant	2×10^{-4}	8×10^{-6}
13	Slightly resistant	8×10^{-5}	6×10^{-5}
13[c]	Slightly resistant	4×10^{-5}	$\leq 4 \times 10^{-5}$
16	Slightly resistant	9×10^{-4}	2×10^{-5}
16[c]	Slightly resistant	3×10^{-4}	2×10^{-5}
18	Medium resistant	7×10^{-4}	7×10^{-4}
18[c]	Medium resistant	2×10^{-3}	$\leq 3 \times 10^{-4}$
TGE900(pSF1E)	Resistant		1.2
			3.1

[a] Log-phase cells of *E. coli* TGE900(pAH12) were thermoinduced as described in Materials and Methods, and survivors after 30 min at 42°C were obtained by plating on LB agar plus ampicillin (100 μg ml^{-1}) and incubation at 28°C. Log-phase cells from surviving clones were again thermoinduced at 42°C for 30 and 60 min.

[b] Classification after incubation overnight at 42°C on agar plates (cells were streaked on LB agar with ampicillin).

[c] Plasmid DNA from the original clone was isolated and used to transform *E. coli* TGE900.

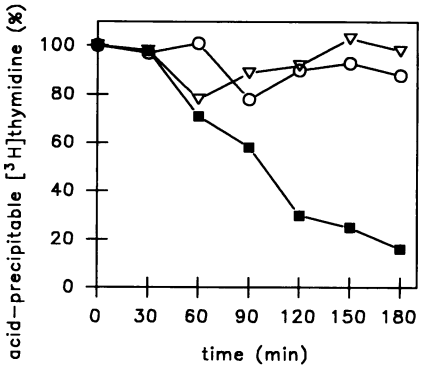

FIG. 3. Intracellular DNA degradation in *E. coli* TGE900(pAH12) following thermoinduction. Cells were labeled with [^{3}H]thymidine at 28°C as described in Materials and Methods. Thermoinduction occurred by shifting cultures to 42°C from time zero to 30 min and then to 28°C from 30 to 180 min. At the times indicated, the amounts of TCA-insoluble radioactive material in the thermoinduced culture (■), in the culture thermoinduced in the presence of chloramphenicol (▽), and in the culture kept at 28°C throughout (○) were determined.

examined, 21 behaved like wild-type *E. coli* TGE900(pAH12), i.e., they grew fully at 28°C, but only a few colonies appeared at 42°C in the streaks. It was concluded that most of the survivors of thermoinduction (84%) were not resistant to the intracellular action of the *Serratia* nuclease and that they were not *nuc*-defective mutants. More colonies appeared at 42°C in the streaks of four clones than in the streaks of TGE900(pAH12). To see whether these four clones had altered plasmids or chromosomal mutations, *E. coli* TGE900 was transformed with plasmid DNA isolated from each of the four clones. The transformants showed the same growth in streaks on LB agar with ampicillin at 42°C as the original surviving clones (Table 3), suggesting that mutations in the bacterial chromosome were not responsible for the survival of these clones. Gel electrophoretic analysis of DNA from the four plasmids showed the same plasmid size as that of wild-type pAH12. Therefore, the presence of large deletions in these plasmids can be ruled out, but not small deletions or point mutations. From the four original clones and four secondary clones, the time course of killing at 42°C in log cultures was determined (Table 3). The killing kinetics of original and secondary clones were similar. Three of the four clones (numbers 8, 13, and 16) showed slower killing kinetics than TGE900(pAH12) (Fig. 2) but reached a low level of survival similar to that of strain TGE900(pAH12) (Fig. 2) after 60 min of incubation at 42°C. One clone showed reduced killing (survival of approximately 7×10^{-4}) compared with that of the wild type (2.3×10^{-5}; Table 2). In summary, 21 of the 25 surviving clones examined were only transiently refractory to thermoinduced killing. It is possible that the p_L promoter in these cells was not induced during the first heat treatment. The other four surviving clones showed slightly decreased sensitivity to heat treatment, resulting in somewhat slower killing kinetics possibly due to mutations located on the plasmid. In none of these 25 clones was thermoinduced killing totally aborted.

Intracellular DNA degradation. Thermoinduction of the gene coding for the leader-free *Serratia* nuclease in *E. coli* TGE900(pAH12) led to the breakdown of intracellular (chromosomal and plasmid) DNA (Fig. 3). In these experiments, cellular DNA was labeled with [^{3}H]thymidine and the fraction of TCA-insoluble radioactivity was determined after various incubation times. Following induction for 30 min at 42°C, DNA was degraded to acid-soluble material for at least 2.5 h (Fig. 3). These data show that the *Serratia* nuclease without the leader peptide is active in the cytoplasm of cells. DNA degradation (and possibly RNA degradation) continued for at least 2.5 h after derepression of the *nuc*ΔL gene, leading to the conversion of over 80% of cellular DNA to acid-soluble material. In cells grown at 28°C all the time or thermoinduced at 42°C with simultaneous addition of chloramphenicol (100 μg ml^{-1}), there was little, if any, DNA degradation.

Following thermoinduction, the optical density of the culture and the size of cells increased somewhat while cell integrity did not change for at least 2 h as seen with a light microscope. It was concluded that although cell viability was lost, lysis of cells did not occur.

The amount of plasmid DNA isolated from TGE900 (pAH12) cells after 30 min of heat induction by the procedure described above was about 30% of the amount isolated from uninduced cells. It did not decline during a further 30-min incubation after induction, suggesting that dead cells retained a fraction of the plasmid DNA. This resembles other previous findings made with maxicells (12).

DISCUSSION

The suicide system presented here consists of the truncated *nuc* gene of *S. marcescens* (deletion of the sequence for a leader peptide) coding for a powerful DNase and RNase cloned downstream of the lambda p_L promoter and controlled by the thermosensitive lambda *c*I857 repressor. Upon thermoinduction, cell killing correlated with DNA degradation, i.e., thermoinduction (30 min at 42°C) led to a minimum of cell survival and to extensive intracellular DNA breakdown. The amount of enzyme produced following induction suffices to degrade the majority of intracellular DNA in the culture to acid-soluble material within 3 h. The intracellular milieu apparently not only provides favorable conditions for the activity of the enzyme but also may contribute to the stability of the enzyme by providing a high concentration of proteins shown to stimulate nuclease activity toward RNA and DNA in

vitro and to stabilize the purified enzyme against thermal inactivation (1). The intracellular activity of the enzyme is notable because the *Serratia* nuclease has two disulfide bonds, which are essential for the activity of the extracellular enzyme (3) and whose formation could be inhibited under the prevailing redox conditions in the intracellular milieu. Recently, it was found that overexpression of the *nuc* gene with the leader peptide-coding sequence in *E. coli* resulted in aggregation and sequestration of the protein in inclusion bodies, from which active leader-free *Serratia* nuclease could be recovered by in vitro procedures (10).

The efficiency of killing by this system (2×10^{-5}) is similar to or better than that of previously published suicide systems not involving a nuclease. For example, efficiencies of 5×10^{-2} (29), 10^{-3} (24), and 10^{-5} to 10^{-6} (7, 14) have been described. When two copies of the killing gene (membrane destruction) were employed, killing of up to 10^{-8} was observed (14). It is not known whether duplication of the nuclease gene would increase killing. The fact that of the 25 survivors examined after thermoinduced killing of TGE900(pAH12), most were as sensitive as the initial strain and the rest were still highly sensitive suggests that the system presented is not very prone to mutational escape events. Knudsen and Karlström (15) described a suicide system based on the *relF* gene of *E. coli* controlled by various *lac* promoters localized on plasmids. They found that surviving clones contained plasmids with mutations in the suicide cassette. In another suicide system, with the *gef* gene product as the killing function (7), 31 survivors, of which 21 (68%) were Gef resistant, were examined. In these survivors, the two plasmids carrying the killing and regulatory elements seemed to be unchanged so the host mechanism to escape killing remained unknown.

It is not yet clear to what extent the nucleolytic killing of cells by this suicide system prevents horizontal gene transfer, although most of the cellular DNA is converted to acid-soluble material following induction. A cell with essentially no chromosomal DNA but retaining a plasmid can serve as a recipient of conjugal transfer and allow mobilization of the retained plasmid for many hours (12). Further experiments are necessary to determine the kinetics of the loss of DNase activity after derepression of the *nuc*ΔL gene and the decay kinetics of transformation-active DNA, including chromosomal DNA and plasmid DNA.

The suicide system based on the controlled expression of a nucleotide sequence-independent nuclease can be adopted for a variety of applications. For instance, for the regulation of cell survival in the environment, the nuclease killing gene could be put under the control of other regulators more relevant to the environment, such as those responding to starvation for specific substances. For use in closed systems, the physiologically induced suicide of cells could substitute for killing by the addition of chemicals. Moreover, since cell lysis does not accompany killing, during the industrial production of substances by GEMs, the cell entity could be preserved while the mass of DNA (and possibly RNA) was degraded, leading to a reduction in the effort needed to remove nucleic acids from the product. For such applications, chemical induction of expression of the truncated nuclease gene may be desirable (such as by isopropyl-β-D-thiogalactopyranoside) if high-temperature regimens have to be avoided because of the thermal sensitivity of the product. Further efforts to reduce the segregation of the plasmid and to improve its killing efficiency are necessary. Also, the functioning of this suicide system in bacteria other than *E. coli* needs to be studied. Several of these possibilities are being examined with a variety of derivatives of the suicide system described here.

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